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Treatment of Experimental Viral Infections with Immunomodulators

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ABSTRACT

The prophylactic or therapeutic antiviral activity of selected immunomodulators was evaluated against bunya-, flavi-, and arenavirus infections. CL-246.738, an acridine hydrochloride derivative (AD) made by Lederle Laboratories, was highly effective against Rift Valley fever virus (RVFV), when given by oral or parenteral routes. S-26308, a quinolinamine derivative (QD) from Riker Laboratories, given orally or parenterally exhibited marked efficacy against RVFV infection in mice. While three doses were required for maximum therapeutic efficacy (85%), a single dose yielded 60% survivors. Viremia was completely abrogated by AD and QD. Human recombinant α-interferon was also very effective for treatment of yellow fever virus infections. The stabilized form of polyriboinosinic: polyribocytidylic acid [poly(ICLC)] was effective in rodents against bunyavirus infections, but not against arenavirus infection. Furthermore, a marked synergism was obtained when AD or poly(ICLC) therapy was combined with the antiviral drug ribavirin for the treatment of RVFV infection.

Reprints

KEYWORDS

Immunomodulators; Antiviral; Interferon; NK Cells; Macrophages; Liposomes.

INTRODUCTION

In the past two decades, interactions between viruses and the immune system have been studied extensively (Allison, 1972; Mims, 1982). These investigations have revealed that many specific and nonspecific immune mechanisms protect the host against viral infection, diminish viral spread, and promote recovery. The relative importance of each mechanism is strongly dependent upon the virus in question (Allison, 1974). Effective treatment of viral infections by stimulation of the specific and nonspecific compartments of the immune systems has been a long standing goal of clinicians and basic scientists alike. As a result of recent advances in understanding the immune system, the use of immunomodulatory compounds to modify the host's biological response is emerging as a new and rational form of antiviral therapy. The purpose of this work was to evaluate the antiviral efficacy of selected immunomodulatory compounds against infection with representative members of three viral families. Previous

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studies have demonstrated the immunomodulatory activity of these compounds (Dietrich, 1986; Fidler, 1982; Levy, 1983; Miller, 1986; Wang, 1986). Our study focused on their antiviral efficacy.

RESULTS

Preliminary studies indicated that administration of a single, 50-mg/kg, oral dose of AD was effective (60 and 70% survivors, respectively) when given either 1 day before or 1 day after challenge of 6-8-week-old, Swiss Webster mice with 250 plaque forming units (PFU) of RVFV. Multiple, daily, 50-mg/kg doses of AD were well tolerated and improved survival to 83% (data are not shown). Because the biological activity of immunomodulators, including AD, is known to last several days and because frequent administration of immunomodulators may cause hyporesponsiveness, it was important to select an effective treatment regimen with the fewest possible injections (Fig. 1). Practically identical efficacy was obtained (80, 90, or 100% survivors, respectively) when a daily, 50-mg/kg, oral dose of AD was given in a five-dose regimen on days 1 to 5; a three-dose treatment regimen given on days 1, 4, and 7; or a four-dose regimen given on days 1, 3, 6, and 10. When initiation of AD treatment was delayed until 48 hr postinfection, 40% of treated animals survived (data not shown). Treatment initiated more than 48 hr postinfection was not effective.

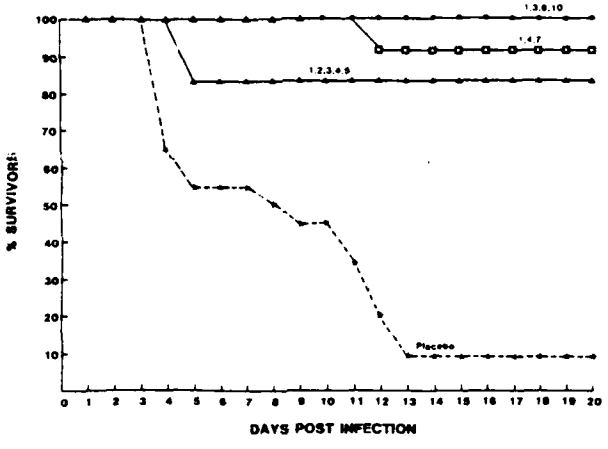

Fig. 1. Efficacy of AD (CL-246.738) against RVFV infection in CD-1 mice. Mice (n = 18) were challenged with 250 PFU of RVFV on day 0 and treated with daily, oral, 50-mg/kg doses of AD given as follows: (▲) on days 1 to 5; (□) on days 1, 4, and 7; or (●) on days 1, 3, 6, and 10. Placebo-treated mice are represented by the dashed line.

A therapeutic synergism was revealed with AD and ribavirin wherein effective treatment of RVFV-infected CD-1 mice was achieved by combining doses of each drug which, when given alone, were not effective (Fig. 2). In this study, 12.5 mg/kg of AD (orally) and 50 mg/kg of ribavirin (intraperitoneally), were given either alone or in combination on days 1, 3, 6, and 8. This combination treatment yielded 80% long-term survivors, whereas no mice survived in those groups given AD or ribavirin alone. The combination treatment given on this

schedule and dose decreased viremia 20-fold, while AD had no demonstrable antiviral effect; ribavirin reduced the viral titer approximately 10-fold.

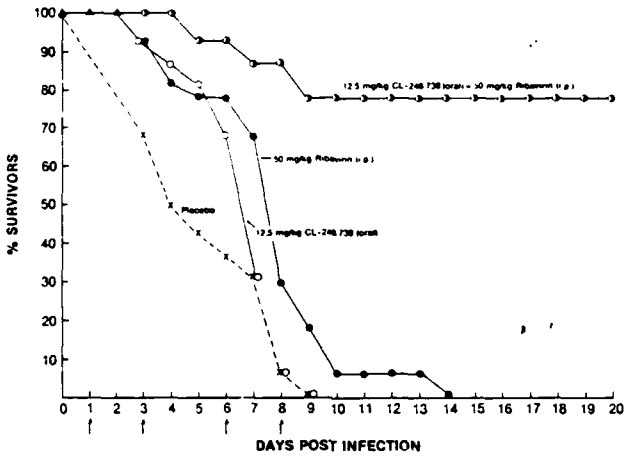

Fig. 2. Therapeutic synergism with AD (CL-246.738) and ribavirin in treatment of RVFV-infected, CD-1 mice. Mice (n = 16) were challenged with 250 PFU of RVFV on day 0 and treated on days 1, 3, 6, and 8 as follows: 12.5 mg/kg of AD given orally (O); 50 mg/kg of ribavirin given intraperitoneally (●); a combination of 12.5 mg/kg of AD orally and 50 mg/kg of ribavirin intraperitoneally (◑); or placebo (X).

Efficacy studies performed with a soluble HCl salt of the quinolinamine derivative (QD) revealed that a single oral dose had considerable antiviral activity when given therapeutically (Fig. 3). In contrast, a single oral dose of QD given prophylactically was marginally effective while repeated doses considerably enhanced the efficacy (data not shown). With repeated therapeutic doses, treatment was effective with three 12.5-mg/kg doses of QD given subcutaneously on days 1, 6, and 11 postinfection, protecting 76% of CD-1 mice from a RVFV challenge (Fig. 4). Oral administration of 12.5- or 25-mg/kg doses was less effective. Viremia was completely abrogated by 12.5 mg/kg of QD given subcutaneously (sc) or orally on days 1, 4, 10 and 13 (data not shown). In RVFV-infected mice, there was a rapid elevation of liver function enzymes and bilirubin values. Normal liver function enzyme and bilirubin values were maintained in RVFV-infected mice treated with 12.5 mg/kg of QD on days 1, 4, 10, and 13. This is illustrated in Fig. 5 by alanine aminotransferase (ALT) serum values. Enzyme levels were elevated in infected-untreated mice, while they remained within the normal range for uninfected-drug control, uninfected-placebo control, and infected-treated mice. Since oral treatment with antiviral drugs is more practical than parenteral administration, we explored the use of three vehicles which also satisfactorily solubilized the free base of QD. Results with two of them, 1% lactic acid and 85% oleic acid, are shown in Fig. 6. While oleic acid may have had a detrimental effect as a vehicle, and/or the compound's solubility was not optimal in that vehicle, lactic acid appeared to be suitable for both oral and sc administration, yielding 68 and 54% survivors, respectively, with administration of 12.5 mg/kg. The therapeutic efficacy of QD was increased when the free base was solubilized in 5% Tween-80; oral

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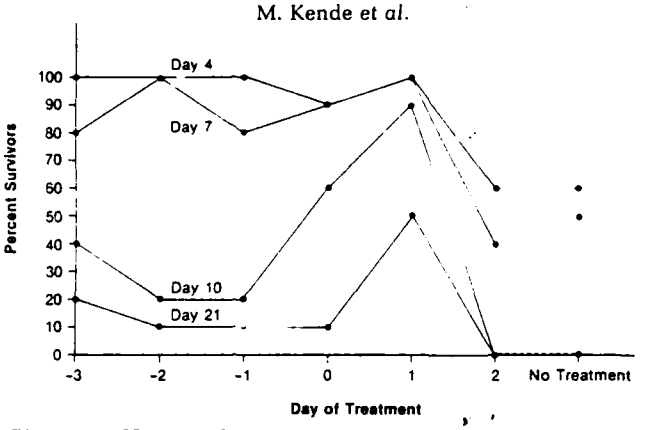

Fig. 3. Efficacy of QD (S-26308) against RVFV infection in CD-1 mice. Mice (n = 10) were challenged with 250 PFU of RVFV on day 0 and treated with a single, oral, 25-mg/kg dose of QD on day -3 to day +2. The number of surviving mice was recorded on days 4, 7, 10, and 21 after each treatment.

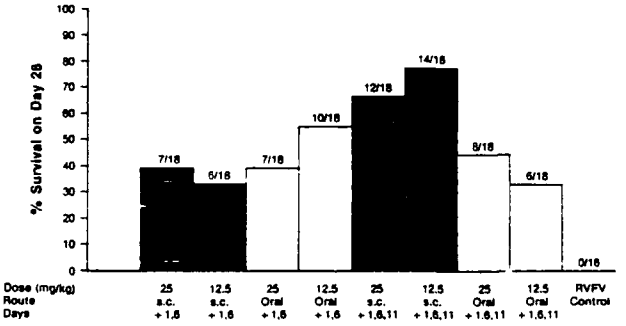

Fig. 4. Efficacy of QD (S-26308) against RVFV infection in CD-1 mice. Mice were challenged with 250 PFU of RVFV and treated subcutaneously (solid blocks) or orally (open blocks) with 12.5- or 25-mg/kg doses of QD given on days 1 and 6 or days 1, 6, and 11 postinfection.

administration of 12 or 6 mg/kg, yielded 82 and 50% survivors, respectively (Table 1). Since the single dose LD_{50} of QD free base in 5% Tween-80 is 500 mg/kg, oral administration of 12 mg/kg of the compound appears to have appropriate safety margin for antiviral therapy.

Muramyltripeptide phosphatidyl-ethanolamine (MTP-PE, Ciba-Geigy, Switzerland), an immunomodulator with antiviral efficacy, is among the few immunomodulators which does not induce interferon (Dietrich, 1986). It is known to stimulate the tumoricidal, cytotoxic reactivity of macrophages and natural killer (NK) cells (Fidler, 1982). Prophylactic or therapeutic administration of as little as 25 ug per mouse of liposome-encapsulated MTP-PE (LE-MTP) was effective in Swiss Webster mice against a relatively small (25 PFU) challenge of RVFV (Fig. 7).

TABLE 1 Efficacy of Quinolinamine in Rift Valley Fever-Infected CD-1 Mice

	Quinolinamine - free base in 5% Tween-80		Quinolinamine - HCl in water	
Dose	12 mg/kg	6 mg/kg	12 mg/kg	6 mg/kg
Treatment days	1,5,9,12	1,3,5,7,9,12	1,5,9,12	1,3,5,7,9,12
% Survivors	82	50	69	33
Median survival time (days)	>21	>21	>21	10.5

N = 16

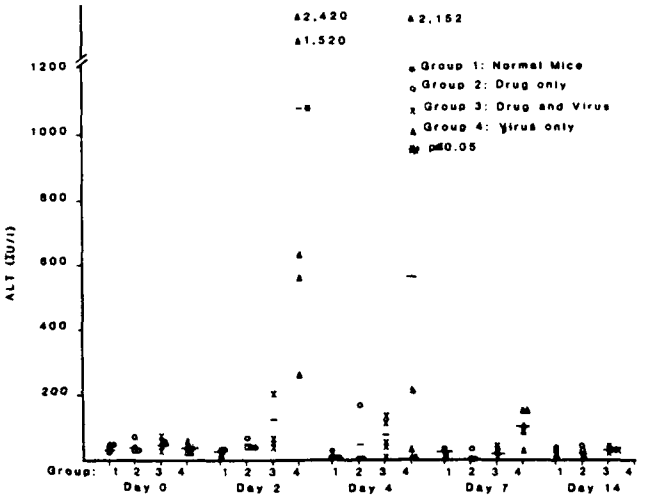

Fig. 5. Alanine aminotransferase (ALT) enzyme values in RVFV-infected, CD-1 mice. Mice were infected with 250 PFU of RVFV on day 0 and treated subcutaneously with 12.5 mg/kg of QD on days 1, 4, 10, and 13.

Significantly more survivors were obtained in the treated group than in the untreated, virus-infected, control group of mice, even when therapy was initiated as late as 3 days postinfection. A control mixture of empty liposomes and MTP-PE had no beneficial effect, regardless of when administered. The liposome-encapsulated MTP-PE we used in our initial studies was prepared in our laboratory according to published methodologies (Fidler, 1982). Since quantitation of MTP-PE in encapsulated liposomes is difficult, it was not employed with every batch, and thus the amounts employed varied considerably, perhaps influencing the outcome of some tests. Therefore, standardized, liposome-encapsulated MTP-PE was produced by Ciba-Geigy Switzerland to eliminate this variable. Such preparations were found to have prophylactic efficacy against 250 PFU of RVFV in Swiss Webster mice when administered intranasally, but were not effective when given intravenously (Fig. 8).

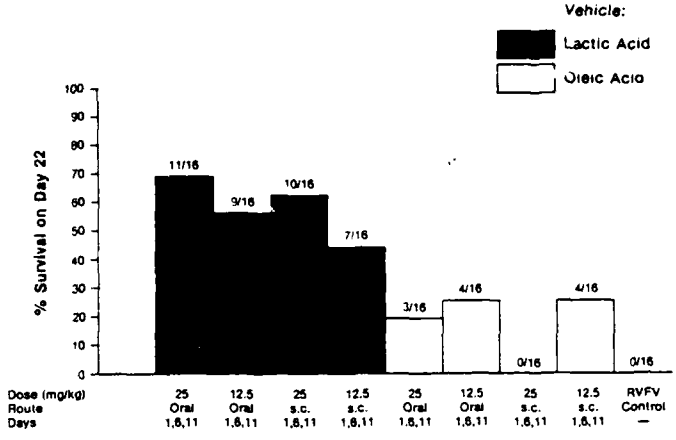

Fig. 6. Efficacy of QD (S-26308) against RVFV infection in CD-1 mice. Mice were infected with 250 PFU of RVFV on day 0 and treated on postinfection days 1, 6, and 11 with oral or subcutaneous administration of 12.5- or 25-mg/kg doses of QD solubilized in lactic acid (solid blocks) or oleic acid (open blocks) vehicles.

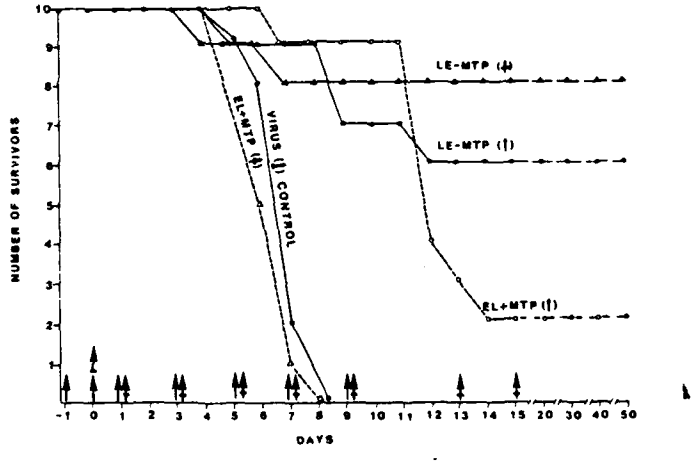

Fig. 7. Prophylactic (↑) and therapeutic (↟) efficacy of liposome encapsulated MTP-PE against RVFV infection in Swiss Webster mice. Mice (n = 10) were infected with 25 PFU of RVFV on day 0 and given 25 µg of liposome encapsulated MTP-PE (LE-MTP) or a mixture of empty liposome and MTP-PE (EL+MTP) by intravenous administration on days indicated by arrows.

Polyriboinosinic-polyribocytidylic acid stabilized with poly-L-lysine and carboxymethylcellulose [poly(ICLC)] is effective in the treatment of a variety of experimental viral infections (Harrington, 1977; Stephen, 1977). We have

reported previously on the efficacy of poly(ICLC) against RVFV infection in Swiss Webster and CD-1 mice (Kende, 1987). The optimal treatment schedule for poly(ICLC) was determined by using the Cox Model (Table 2), which ranks treatment efficacy in terms of relative risk of death (Hopkins, 1983). For this evaluation, groups of mice were treated with a one-dose, two-dose, or three-dose regimen of 20 µg of poly(ICLC) given ip by daily injections before or after viral challenge, as indicated in the four left columns. The relative risk of death for the various treatment regimens was ranked according to increasing or decreasing values as compared to the standard treatment value, defined as 1 for the day 0 treatment. Table 2 is divided into three sections, with the upper third containing treatment schedules which produced efficacies inferior to the standard (day 0) treatment; the middle third contains schedules with equal efficacy; and the lower third contains schedules that were significantly better than the standard treatment. These three efficacy categories were distinct because the relative risk of death associated with the treatment was significantly higher ($p < 0.05$), indistinguishable ($p \geq 0.05$), or significantly lower ($p < 0.05$) than the day 0 treatment. The efficacy obtained on days -1 and +1 was undefined because of the proximity of the two doses. The optimal treatment schedule was a three-dose regimen given on days -4, +1, and +6. This schedule induced the longest duration of effect concurrent with highest efficacy. Additional studies demonstrated comparable efficacy with 1, 5, and 10 µg doses of poly(ICLC) administered on the optimal treatment schedule (data not shown).

Table 2 Efficacy Ranking with Incremental Relative Risk of Death of 20 µg poly(ICLC) Treatment Regimens Versus Standard (Day 0) Treatment in RVFV-Infected Mice (N = 10)

TREATMENT ON DAY (s)				NO. OF SURVIVORS	RELATIVE RISK OF DEATH	P VALUE
+3				0	38.07	$< 0.05^{*}$
+2				0	21.19	
	-5			0	7.81	
		-4 +4		0	6.42	
	-3			0	4.96	
		-5 +5		1	4.47	
	-4			1	4.33	
		-3 +3		0	4.12	
	-2			1	2.69	
+1				1	2.39	$\geq 0.05^{**}$
		-2 +2		2	1.35	
		-3 +1		2	1.14	
		-2 +2		3	1.06	
	0 (STANDARD TREATMENT)			3	1.00	
	-1			4	0.92	
			-1 +6 +11	7	0.39	
		-1 +4		8	0.36	
			-2 +2 +4	8	0.22	
		0 +4		8	0.21	$< 0.05^{***}$
			-3 +1 +6	8	0.21	
			-1 +1 +8	9	0.11	
			-4 +1 +6	9	0.11	
			0 +4 +7	9	0.10	
		-1 +1		10	UNDEFINED	

$P < 0.05^{*}$ = MORE ANIMALS AT RISK THAN WITH STANDARD TREATMENT

$P \geq 0.05^{**}$ = INDISTINGUISHABLE

$P < 0.05^{***}$ = LESS ANIMALS AT RISK

= SIGNIFICANTLY LESS AT RISK

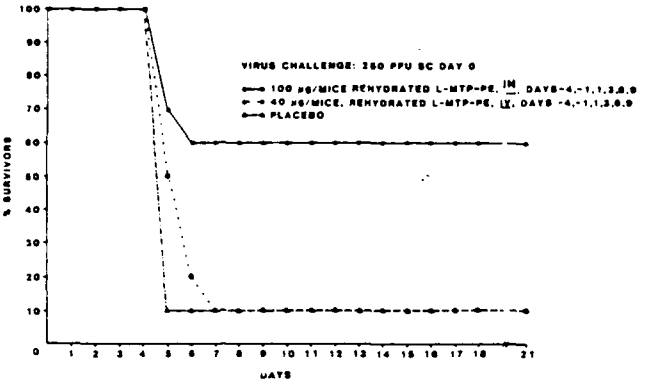

Fig. 8. Efficacy of liposome encapsulate MTP-PE (LE-MTP) *against* RVFV *infection* in *Swiss* Webster mice. Mice (n = 10) were given 250 PFU of RVFV on day 0 and treated with (——) 100 µg of LE-MTP intranasally; (– –) 40 µg of LE-MTP intravenously; or (——) placebo.

The therapeutic efficacy of poly(ICLC) in treatment of RVFV-infected mice is shown in Fig. 9. Groups of mice were treated with 8-, 7-, or 6-dose regimens of 20 µg of poly(ICLC) per mouse, commencing respectively 1, 2, or 3 days after inoculation with RVFV. The treatment series was concluded 16 days after challenge yielding 100, 40, and 10% survivors, respectively. Most untreated mice died by day 5, and all by day 10.

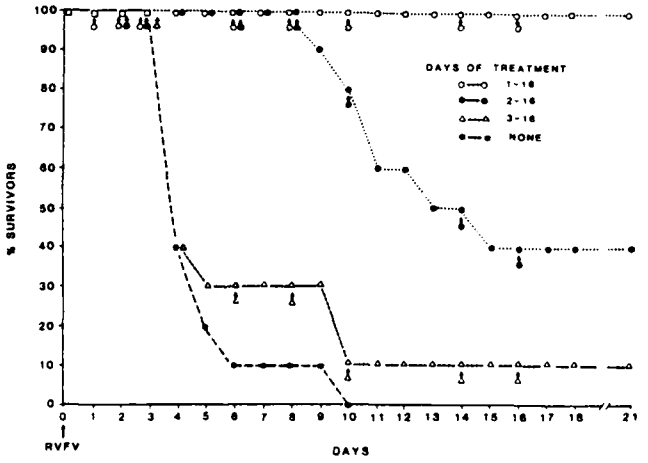

Fig. 9. Therapeutic efficacy of poly(ICLC) against RVFV infection in Swiss Webster mice. Mice (n = 10) were challenged with 250 PFU of RVFV on day 0 and treated intraperitoneally with 20 µg of poly(ICLC) per mouse given in 8 (○), 7 (●), or 6 (▲) daily doses commencing on days 1, 2, or 3. Days of treatment are indicated by arrows.

Therapeutic treatments with poly(ICLC) doses lower than 20 μg were less effective or not effective at all (data not shown). We next examined the possibility of synergism when using ineffective doses of poly(ICLC) together with ribavirin during the period between the time of virus inoculation and death (Fig. 10). Challenge with 250 PFU of RVFV caused death in 92% of the untreated mice by day 6. Initiation of treatment 24 hr after challenge with 1 μg of poly(ICLC) or 50, 25, or 12.5 mg/kg of ribavirin, resulted in 17, 33, 8, and 0% survivors, respectively. However, with the combination treatment, a high degree of synergism was revealed when 1 μg poly(ICLC) was combined with 50, 25, or 12.5 mg/kg of ribavirin, yielding 75, 92, and 58% survivors, respectively.

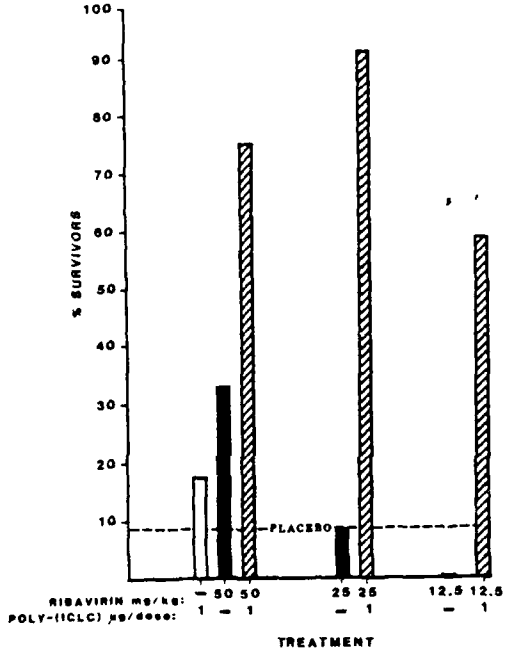

Fig. 10. Enhanced therapeutic efficacy of poly(ICLC) and ribavirin against RVFV infection in mice on day 21. Mice were challenged with 250 PFU of RVFV on day 0 and treatment was initiated 24 hr postinfection as follows: (□) 1 μg of poly(ICLC) per mouse on days 1, 4, and 9; (■) 50, 25, or 12.5 mg/kg of ribavirin on days 1 to 4, 7, 9, and 11; (▨) 1 μg of poly(ICLC) per mouse on days 1, 4, and 9 plus 50, 25, or 12.5 mg/kg of ribavirin on days 1 to 4, 7, 9, and 11; and (dashed line) placebo.

Prophylactic treatment of RVFV infected mice with three doses of 20 or 1 μg of poly(ICLC), given with 3 to 4 day intervals between injections consistently gave 80 to 100% survivors. Consequently, these doses and schedule were employed for the evaluation of the compound's interferon-inducing potency. Compared with 20 μg of poly(ICLC) per mouse, 1 μg induced less interferon, viz. 6000 IU and 1000 IU/ml, respectively (data not shown). Apparently, 1000 IU/ml was sufficient to protect 80 to 100% of the infected mice from RVFV infection.

As the primary replication site for several viruses is the liver, we examined the effect of poly(ICLC) in stimulating liver NK cells (Table 3) and macrophage cells (Table 4) in cytotoxicity assays. In this study 1 mg/kg [~20ug poly(ICLC)] stimulated NK and macrophage cytotoxicity. Nonspecific cellular reactivity probably plays a role in the stimulation of the antiviral state of the host.

TABLE 3 In Vivo Activation of Liver NK Cell Cytotoxicity of C_3H/HeN Mice

Activator[a]	Dose	% Cytotoxicity 50:1	% Cytotoxicity 25:1
HBSS	0.2 ml	40.1	19.9
Poly(ICLC)	1.0 mg/kg	80.5[b]	65.1[b]
C. parvum	0.8 mg/kg	79.7[b]	71.9[b]

[a]The activator was injected intraperitoneally 3 days prior to the assessment of NK cell cytotoxicity using a 4-hr ^{51}Cr release assay with YAC tumor cells.

[b]Significant increase in cytotoxicity ($P < 0.01$)
HBSS = Hanks' balanced salt solution

TABLE 4 In Vivo Activation of Liver Macrophage Cytotoxicity of C_3H/HeN Mice

Activator[a]	Dose	% Specific lysis 50:1	% Specific lysis 25:1
HBSS	0.2 ml	1.1	0.3
Poly(ICLC)	1.0 mg/kg	19.8[b]	17.2[b]
C. parvum	0.8 mg/kg	28.0[b]	22.9[b]

[a]The activator was injected intraperitoneally 3 days prior to the assay with ^{111}In-labeled L-51 tumor target cells.

[b]Significant increase in % specific lysis ($P < 0.01$).

The efficacy of endogenously induced interferon was equaled with exogenous interferon. Therapeutic administration of human recombinant α-interferon (Hoffmann-La Roche) entirely abrogated viremia in African green monkeys given yellow fever virus (Fig. 11). All treated monkeys (n = 4) became seropositive but their yellow fever virus antibody titers were 30 times lower than in placebo-treated animals (n = 4).

Only Pichinde virus infection (used as a model to study the highly pathogenic Lassa fever virus) did not respond to treatment with immunomodulators. However, an enhanced therapeutic response was obtained when a combination of poly(ICLC) and ribavirin was given to strain-13 guinea pigs infected with a lethal challenge of Pichinde virus (Table 5). Combination treatment with ribavirin and AD or QD did not yield enhanced efficacy in Pichinde virus-infected guinea pigs. While combinations of AD or poly(ICLC) and ribavirin were highly synergistic in treating RVFV-infected mice (Fig. 2 and Fig. 10 respectively), combination of QD and ribavirin was only marginally synergistic.

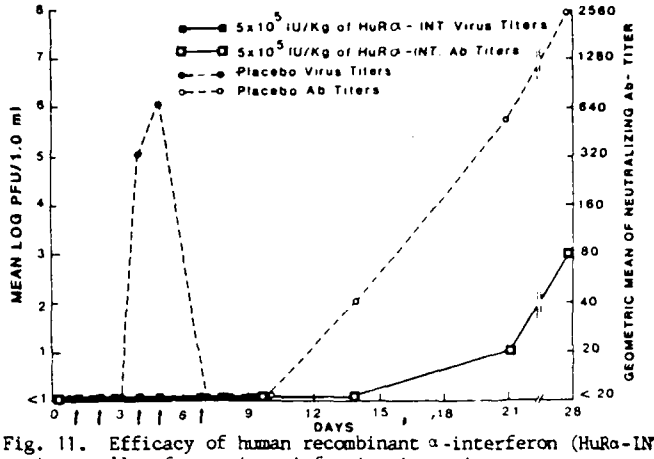

Fig. 11. Efficacy of human recombinant α-interferon (HuRα-INT) against yellow fever virus infection in African green monkeys. Monkeys were challenged with 400 PFU of Dakar strain yellow fever virus on day 0 and given 5 x 10^5 IU/Kg of HuRα-INT (■) or placebo (●) on days 1, 2, 4, 5, and 7.

TABLE 5 Enhanced Therapeutic Efficacy of Ribavirin and Selected Immunomodulators Against Rift Valley Fever and Pichinde Virus Infections

Virus-host	Ribavirin + Poly(ICLC)	Ribavirin + Acridine (AD)	Ribavirin + Quinolinamine HCl (QD)
Rift Valley fever virus S-W or CD-1 Mice	++++	++++	++
Pichinde virus Strain-13 guinea pig	++++	0	0

0 = No survivors. ++ = 25-50% survivors. ++++ = 76-100% survivors.

DISCUSSION

The antiviral efficacy of selected immunomodulators was established against viral infections in several animal species. The antiviral activity of immunomodulators is exerted by their ability to stimulate humoral and cellular immune responses nonspecifically. For that reason, their efficacy does not depend on the inhibition of viral specific enzymes which may not be common to many or all of the viruses. The use of a broad spectrum, nonspecific immunomodulator would be advantageous for antiviral treatment when the identity of the virus is unknown, or when the properties of the virus are altered. Furthermore, this group of substances is also suitable for general antiviral protection when vaccination is not possible before entering an endemic area. All but one of the immunomodulators examined in this study are potent interferon

inducers. Because recombinant interferon is available, one question that remains is whether its use is preferable to that of interferon inducers. While comparative studies are required to decide which is more effective and/or safer, induction of antibodies against interferon can occur only with the use of exogenous interferon. For this reason, an interferon inducer appears to be more suitable for prophylactic treatment.

Interferon is not the sole mediator of nonspecific, antiviral, immune responses. Interferon can serve to induce other soluble mediators (cytokines), and itself can be induced by cellular products, which operate mainly as intercellular signals. In either case, a series of interactions occur between the soluble mediators, macrophages, and various lymphocyte subsets resulting in the activation of nonspecific, antiviral, immune mechanisms. The cytokines are produced in very small quantities, but are extremely potent in their biological activities. Because of recent advances in molecular biology and recombinant technology, cytokines like interleukin 1 and 2 are available in sufficient quantity to study their possible antiviral role. So far, interleukin 1 and 2 have failed to achieve the expected efficacy.

It is conceivable that prophylactic-antiviral activity giving continuous protection against viral infection can be maintained for a prolonged period by administration of immunomodulators at 4- to 6-day intervals. Therapeutic administration of immunomodulators can be very valuable, and additional therapeutic advantages can be obtained by combination of immunomodulators with effective antiviral drugs such as ribavirin. Enhancement of therapeutic efficacy by combination therapy with compounds having different modes of action offers an attractive approach for treatment of human diseases. Additional advantages can be gained by carrier-mediated delivery of immunomodulators or antivirals, as treatment with these preparations requires smaller doses to achieve the same efficacy obtained with "free" drug (Kende, 1985).

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